Electrophoretic Fractionation of the Various Subunits from Heat-Denatured Insoluble Collagen

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The collagen subunits are recognized in denatured soluble collagens [1] which have been fractionated either by chromatography in a carboxymethylcellulose column [2] or by gel electrophoresis.[3] Some commercial gelatins contain remnants of these subunits and certain extracts of insoluble collagen yield column chromatographic patterns which resemble those from soluble collagen.[4,5] We report here the gel electrophoretic demonstration of the various collagen subunits in the extracts from *insoluble* collagen.

The skins of adult guinea pigs (weighing in average 650 g) were cleaned from hair and subcutaneous fat, minced in a meat grinder and homogenized with a homogenizer of a revolving-blade type (No. 21 00 00, E. Bühler, Tübingen, West Germany) into cold 0.45 M sodium chloride. The neutral salt-soluble collagen was removed by three subsequent extractions with the 2-fold volume of 0.45 M NaCl overnight.[6] The supernatants were removed by centrifugation in MSE refrigerated centrifuge (17 000 rev./min for 120 min). The acid-soluble collagen was removed analogously with nine extractions under shaking with 0.15 M, pH 3.7, citrate buffer.[7] All preparations were carried out in a cold room (+ 4°C).

The insoluble residue was suspended in 0.01 M, pH 4.8, acetate buffer and shaken overnight. The supernatant was removed and the procedure repeated several times, until the supernatant did not foam any more, and its pH remained at 4.8.

The residue was suspended in the 4-fold volume of the 0.01 M, pH 4.8, acetate buffer and gelatinized at + 40°C for 15 min. The supernatant was collected by centrifugation at room temperature and analyzed with starch gel electrophoresis as described previously.[3,8]

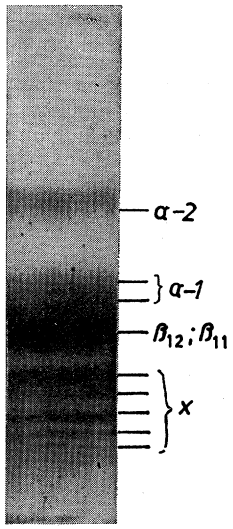

Fig. 1. Starch gel electrophoretic pattern of an extract (15 min at + 40°C) from insoluble collagen of guinea pig skin. The resolution of the β-components cannot be observed in this case.

The result is shown in Fig. 1. The regular subunits are well preserved, but the β-band and "x"-bands (presumably larger aggregates) dominate. In these figures the splitting of the α-1-band can be observed which has also been found in the samples prepared from acid-soluble collagen (V. Näntö, unpublished work from our laboratory).

Insoluble collagen consists thus of two fractions: (1) the first is solubilized as more or less intact subunits, when the secondary structure is denatured, and (2) the second is solubilized as smaller fragments only when the primary structure also is destroyed at higher temperature.

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